Original Research Article

Occurrence and Antibiotic Resistance profiles of Metallo-beta-lactamase-producing *Pseudomonas aeruginosa* among clinical isolates in Enugu Metropolis

ABSTRACT

Introduction: Antibiotic resistance is rising worldwide, posing a challenge for contemporary medicine. Metallo-beta-lactamases (MBLs) confer resistance to beta-lactam antibiotics other than monbactams. It threatens public health because of its vast scope of action and quick spread. The study was undertaken to assess the occurrence of MBL in clinical isolates of *Pseudomonas aeruginosa* in the Enugu metropolis and to determine their resistance profile. **Material and Methods:** The work was conducted from October 2020 to July 2021 in the microbiology laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla. A total of 127 non-duplicate bacterial isolates recovered from clinical samples including wounds, urine, sputum, ear discharge, and catheter tip processed in the microbiology laboratory of four referral hospitals within the Enugu metropolis was used for the study. Isolates were identified and characterized using standard microbiology protocols. Antimicrobial susceptibility was done using the Kirby-Bauer disc diffusion method. Phenotypic detection of Metallo-beta-lactamase production was done using the Combined Disk diffusion Test (CDDT). **Results:** Of the 127 isolates, 68 (53.5%) were resistant to imipenem. Among these, 35 strains were positive for MBL production while 33 isolates were non-MBL producers. The highest prevalence of MBL producers was recorded from wound swabs (20 strains) followed by urine 11 strains. Both MBL producers and non-MBL producers displayed high resistance to most of the antibiotics used except Aztreonam. **Conclusion:** The overall occurrence of MBL among *Pseudomonas aeruginosa* in our study was found to be 27.6%. MBL-producing strains showed higher resistance than the non-MBL-producing strains. Aztreonam was the most potent antibiotic. The most effective approaches to combating this organism include early detection, stringent antibiotic regimens, and adherence to infection control measures.

Keywords: Antibiotic resistance, Carbapenemase, Metallo-beta-lactamase, Phenotypic detection, Combined disc diffusion Test, Pseudomonas aeruginosa

1. Introduction

Pseudomonas aeruginosa is an opportunistic organism that is known to cause nosocomial infections and different diseases such asurinary tract infection, pneumonia, sepsis,and soft-tissue infections [1]. The greatest threat challenging the healthcare sectors in developing nations like Nigeria is the rise and spread of multidrug-resistant (MDR) microorganisms [2]. MDR *P. aeruginosa* infections result in considerable morbidity and mortality. *P. aeruginosa* has a very high level of intrinsic and acquired resistance to numerous antibiotics, making it difficult to treat and limiting therapy options. *P. aeruginosa* exhibits practically all known resistance mechanisms; nevertheless, enzyme production is the predominant mode of acquired resistance, particularly beta-lactamase production [1]. Reliable susceptibility evaluation, cautious use of currently available antibiotics, and assessment of the prevalence of MDR are necessary for reducing the potential challenges associated with diseases caused by multidrug-resistant strains (2). Carbapenems are the last resort for the treatment of *P. aeruginosa* infections. Nevertheless, the high rise in carbapenem resistance causes grave concern in the management of infections caused by *P. aeruginosa*[3]. The World Health Organization (WHO) has recognized the 12 most frequent microorganisms that threaten human health. Carbapenem-resistant *P. aeruginosa* has been identified as one of the most critical, posing a major challengeto patients who need catheters and ventilators [4]. Beta-lactamases are divided into four classes: A, C, and D that uses a serine-based mechanism and metallo-β-lactamase (MBL), while class B needs a bivalent metal ion to function [5].

Metallo-lactamases (MBLs) are a class of carbapenemases that are highly important to healthcare because of their potential to spread over the world and the resulting restricted number of available therapeutic alternatives [6]. These class B carbapenemases require zinc or another heavy metal to catalyze their reactions. MBLs have a wide spectrum of substrates that can catalyze the hydrolysis of almost all beta-lactam antibiotics [7]. Moreover, they are prevented by chelating compounds like ethylenediamine tetraacetic acid (EDTA) [2]. The rising incidence of *P. aeruginosa*-acquired MBL is very disturbing and constitutes a major threat during therapy and infection management [8]. MBL-producing microorganisms can hydrolyze penicillin, cephamycins, cephalosporins, and carbapenems, but not monobactams [9].*P. aeruginosa* bacteria that produce MBL also have antibiotic resistance genes for other classes of antibiotics. MBL genes are found in mobile genetic elements such as plasmids, transposons, integrons, or linked with insertion sequences, and they can move within and between species. [9].

Antibiotic treatment for serious infections caused by MBL-producing *P. aeruginosa* usually results in a poor clinical outcome. With a worldwide increase in incidence, early detection is critical for establishing effective antibiotic regimens and infection control practices. PCR-based genotyping remains the gold standard for MBL detection and classification; nevertheless, diagnostic laboratories still need to do culture-based phenotypic testing to promptly identify MBL activity [10]. The MBL enzyme can be detected phenotypically in pathogenic organisms. The acknowledged methods include the Modified Hodge Test (MHT), Double-Disc Synergy Test (DDST), Combined Disc Diffusion Test with Imipenem and EDTA (CDDT), and MBL E-Test [11, 12]. Keeping this in mind, the current study was conducted to determine the percentage occurrence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains in our setting, as well as their antibiotic sensitivity pattern, so that an appropriate infection management plan and antibiotic policy can be developed to prevent their spread.

2. MATERIALS AND METHODS

2.2 Study Design

This study was carried out from October 2020 to July 2021 in the microbiology laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla in Enugu State, Nigeria

2.2 Study Area

Enugu Metropolis is located in Enugu State, Nigeria's South-East geopolitical zone. It is surrounded by the states of Kogi and Benue to the north, Ebony to the east, Abia to the south, and Anambra to the west. It is situated at the foot of the Udi Plateau. Enugu State has 17 local governments. The Metropolis has four Tertiary Health Care Centers: the University of Nigeria Teaching Hospital Enugu (UNTH), the Enugu State University Teaching Hospital (ESUTH), the National Orthopedic Hospital Enugu (NOHE), and the Federal Neuropsychiatric Hospital Enugu (FNHE). They function as referral and specialist centers for the southeast geopolitical zone. This was a descriptive cross-sectional research. A total of 127 Pseudomonas aeruginosa isolates were recovered from various clinical samples, including wounds, swabs, urine, sputum, ear discharge, and catheter tips from the University of Nigeria Teaching Hospital (UNTH), National Orthopaedic Hospital, Parklane Hospital, and the Enugu Metropolis Division Hospital.

2.3 Collection of Sample

Isolates previously processed in the microbiology laboratories of the University of Nigeria Teaching Hospital Enugu (UNTH), Enugu State University Teaching Hospital (ESUTH), National Orthopedic Hospital Enugu (NOHE), and some private laboratories in Enugu Metropolis were collected randomly and aseptically and inoculated on nutrient agar slants. The isolates were obtained from urine, wounds, ear discharge, and catheter tips. They were taken to the Microbiology Laboratory at the University of Nigeria Teaching Hospital, Enugu.

2.4 Isolation of Bacteria

All isolated organisms were transported to the laboratory and maintained in nutrient agar slants. They were sub-cultured unto nutrient agar and incubated at 37^0 C for 24 hours. The isolates were sub-cultured onto blood agar, MacConkey (Central Drug House (P) Ltd), and Centrimide agar plates (HiMedia, MH024, IND) and incubated at 37^0 C for 24 hours.

2.5 Identification of Bacteria Isolates

The isolates were identified using growth on Centrimide, colonial morphology, odor, Gram stain, oxidase test, sugar fermentation, and IMViC reactions [13].

2.6 Antimicrobial Susceptibility Testing

The antibiotic susceptibilities of the isolates were evaluated using Kirby Bauer's disk diffusion method, as specified in the Clinical Laboratory Standard Institute (CLSI) guidelines [14]. The antimicrobial used were Imipenem (10µg), cefoxitin (30µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin/clavulanate (20/10 µg), nitrofurantoin (50 µg), ceftazidime (30 µg), cefixime (30 µg), cefotaxime (30 µg), and cefuroxime (30 µg). Mueller-Hinton agar plates were inoculated with a standardized suspension of the isolates comparable to 0.5 McFarland turbidity standards, and the antibiotics discs were aseptically placed on the agar plates. The plates were then incubated at 37 °C for 18 to 24 hours. The Inhibition Zone Diameter (IZD) was measured and recorded following incubation by CLSI standards [15].

2.7 Screening for carbapenemase production

MBL-producing E. coli and K. pneumoniae resistant to imipenem were evaluated for MBL production. Isolates that had reduced susceptibility to imipenem with an inhibition zone diameter of ≤ 21 mm were used as cut-off values according to CLSI guidelines [14].

2.8 Detection of metallo–beta-lactamase production

2.8.1 Combined Disc Diffusion Test (CDDT)

MBL production was detected using the Combined Disc Diffusion Test as per Franklin *et al* with slight modifications [16]. Two 10µg imipenem discs (one impregnated with 10µl of 750µg EDTA Sigma Chemicals, St. Louis, MO) were placed at a distance of 25mm apart on the Mueller Hinton (MH) agar medium inoculated with test organism standardized with 0.5 McFarland standards. After 24 hours of incubation at 37^{o}C, inhibition zones around imipenem and imipenem + EDTA discs were compared. MBL production was seen to have occurred when the zone diameter around the imipenem + EDTA discs increased by more than 4mm as compared to imipenem alone. Before being added to the antibiotic disk(s), EDTA was tested on the isolate(s) alone to ensure that it did not inhibit the test organism and provide a false positive [2].

2.7 Statistical Analysis

All statistical analyses were carried out using SPSS for Windows version 22 (SPSS, Chicago, IL, USA). Descriptive statistics were employed to describe categorical variables (frequency and percentage). The Chi-square (X^2) test (at 95% confidence intervals) was used to compare variables. A p-value of ≤ 0.05 was taken as statistically significant.

2. RESULTS

In Table 1, results show that out of the 127 *Pseudomonas aeruginosa* isolated from diverse sample sources, wound swabs provided the highest number of *Pseudomonas aeruginosa* isolates with a total of 71 (55.9%), followed by Urine: 36 (28.3%) and Sputum: 10 (7.9%). Ear discharge produced 7 (5.5%) isolates of *Pseudomonas aeruginosa,* while the catheter tip produced only 3 (2.4%).

Out of the 127*P.aeruginosa*isolates, 68 (53.5%) were imipenem-resistant. Of the 68 (53.5%) imipenem-resistant isolates, 35 (51.5%) produced MBL, while 33 (48.5%) did not produce MBL.

The highest rate of imipenem resistance was found in catheter tips 66.7% (2/3), followed by urine 63.9% (23/36), and the lowest ear discharge 42.4% (3/7). There was no statistical significance between the source of the isolates and imipenem resistance p= 0.435.

Fig 1 shows the distribution of MBL and Non-MBL producers among the isolates. The highest occurrence of MBL was again obtained from catheter tips 33.3% (1/3) while the lowest was from ear discharge 14.3% (1/7). There was no statistical significance association between the source of isolates and MBL production p= 0.252.

Table 2 shows the antimicrobial susceptibility profile of MBL and non-MBL-producing strains. Among the MBL-producers, the most potent drug was Aztreonam 54.3% followed by Fluoroquinolones (ofloxacin and ciprofloxacin) 42.9% and the least was aminoglycosides (gentamicin) 40%. MBL producers were 100% resistant to Augmentin, nitrofurantoin, ceftazidime, cefixime, cefuroxime, and cefoxitin. Non-MBL-producers were highly susceptible to Aztreonam at 87.9% and were moderately susceptible to fluoroquinolones (ofloxacin and ciprofloxacin) at 60.6% respectively and gentamicin at 57.6%. They were also 100% resistant to Augmentin, nitrofurantoin, ceftazidime, cefixime, cefuroxime, and cefoxitin.

Table 1:Distributionof MBL according to the sources of isolates, imipenem resistance, and MBL-production

Sample	No of isolate/%	No/% Resistant to Imipenem
Wound swab	71 (55.9)	35 (49.3)
Urine	36 (28.3)	23 (63.9)
Sputum	10 (7.9)	5 (50.0)
Ear discharge	7 (5.5)	3 (42.9)
Catheter tip	3 (2.4)	2 (66.7)
Total	**127**	**68 (53.5)**
P = 0.435		

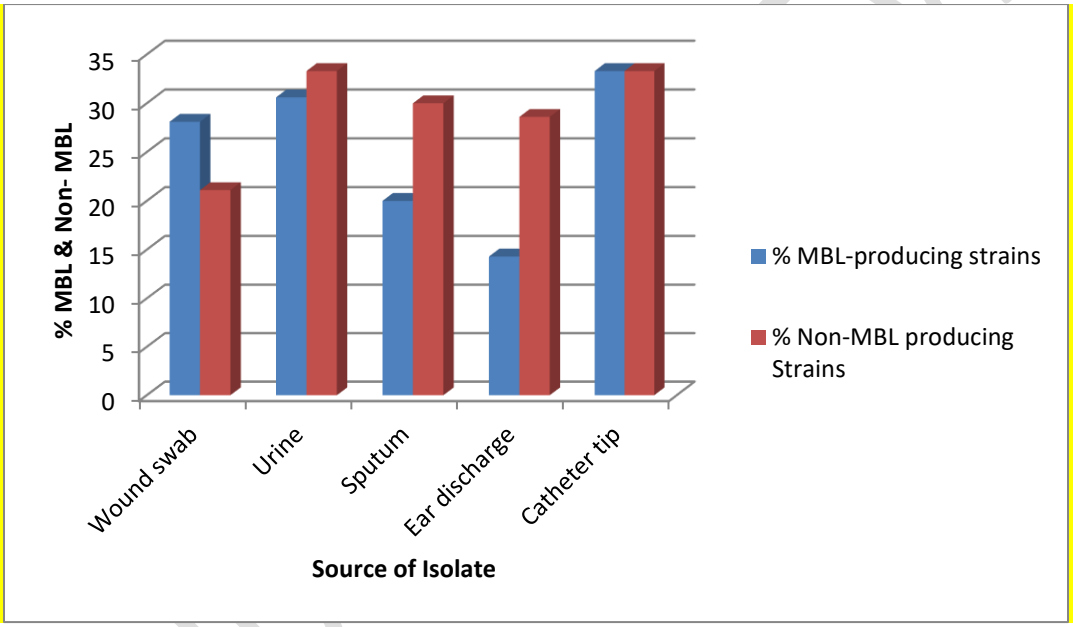

Fig 1: Percentage distribution of MBL and Non-MBL according to the source of isolates

Table 2: Antimicrobial Susceptibility Tests of MBL-producing and Non-MBL-producing *P. aeruginosa* strains

Antibiotics	MBL (% Susceptible)	MBL (% Resistant	Non-MBL (% Susceptible	Non –MBL (% Resistant)

Ofloxacin	15(42.9)	20 (57.1)	20 (60.6)	13 (39.4)
Ciprofoxacin	15 (42.9)	20 (57.1)	20 (60.6)	13 (39.4)
Gentamicin	14 (40.0)	21 (60.0)	19 (57.6)	14 (42.4)
Aztreonam	19 (54.3)	16 (45.7)	29 (87.9)	4 (12.1)
Augmentin	0 (0.0)	35 (100)	0 (0.0)	33 (100)
Nitrofurantoin	0 (0.0)	35 (100)	0 (0.0)	33 (100)
Ceftazidime	0 (0.0)	35 (100)	0 (0.0)	33 (100)
Cefixime	0 (0.0)	35 (100)	0 (0.0)	33 (100)
Cefuroxime	0 (0.0)	35 (100)	0 (0.0)	33 (100)
Cefoxitin	0 (0.0)	35 (100)	0 (0.0)	33 (100)

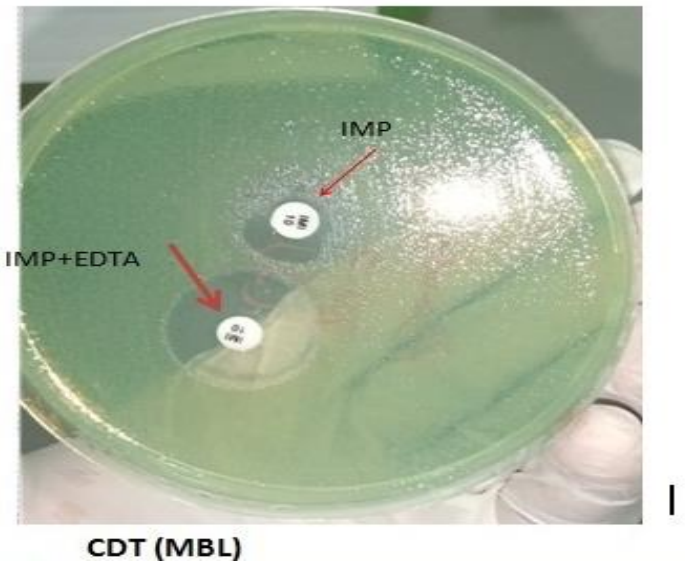

Fig 2: Imipenem-EDTA combined Disc Test

4. DISCUSSION

Metallo-β-lactamase-producing*Pseudomonas aeruginosa* (MPPA) is a significant nosocomial pathogen resistant to all β-lactam antibiotics except monobactams. This has been reported across many nations [17]. Carbapenems are beta-lactam antibiotics that are regarded as the most effective drugs for treating

multidrug-resistant pseudomonas infections due to their stability against the majority of beta-lactamases and their high rate of penetration into bacterial outer membranes (18). Carbapenem hydrolyzing Metallo beta-lactamases can hydrolyze carbapenem and it is an important mechanism for imipenem resistance [19]. The resistance to imipenem in our study was 53.5% (68/127) of the total isolates of P*. aeruginosa.* Comparable rates were found in Iran at 57.1% [20] and in Egypt at 62.5% [21]. Lower rates had been reported in Saudi Arabia at 19.0% [22], in Ethiopia at 22% [23], in Japan at 28.5% [24], and in Taiwan at 16.0% [25]. However, higher rates had been reported in China 77.5% [1], India 74.19% [26], Egypt 78.3% [27], Nepal 79.6% [28], and Pakistan 80.7% [29]. Global variations in *P. aeruginosa* resistance rates could be attributed to differences in antibiotic prescribing methods and sample size around the world.

Our study showed that of the 68 imipenem-resistant isolates of *P. aeruginosa*, 35 (51.5%) were positive for MBL and this aligns with the work of Wang and Wang in China who reported 55.2% [1]. Shrestha et al reported a lower rate of 40.3% in Nepal [28] and Sarjan et al recorded a higher rate of 68% in India [30]. The presence of MBL-positive isolates constitutes not only a therapeutic problem but also an urgent challenge to infection control management. Given that these organisms are not easy to detect, they pose a major threat, particularly due to their role in unsuspected spread throughout the institution and the possibility of participating in horizontal MBL gene transfer with other bacteria in the healthcare facility [30]. This study also recorded the highest occurrence of MBL-producers from catheter tips 33.3% (1/3). This agrees with the work of Devi et al and Viswamohanan et al [18, 30]. However, Wang et al and Sood et al recorded the highest occurrence in respiratory secretions/sputum [1,17] and Sajjan et al and Kali et al reported highest occurrence in wound swabs [30, 32]. The observed highest occurrence in catheter tips in our study may have been due to the ability of *Pseudomonas aeruginosa* to form biofilms on indwelling medical devices thereby increasing its resistant potential.

In our study, MBL-producing isolates recorded 100% resistance to Augmentin, Nitrofurantoin, Ceftazidime, Cefixime, Cefuroxime, and cefoxitin. Many of the strains also displayed high resistance to aminoglycosides (gentamicin) 60% followed by fluoroquinolones (ofloxacin and Ciprofloxacin) 57.1% respectively. MBL genes are carried on mobile genetic elements that also encode resistance genes to aminoglycosides and fluoroquinolones. This justifies the concomitant high resistance rates to these two types of antibiotics, in addition to their production [30]. The most potent drug against MBL producers in our study was Aztreonam with an average sensitivity of 54.3%. Wang and Wang recorded Colistin as the most potent antibiotics but we did not use colistin in our panel of antibiotics. In theory, MBLs can hydrolyze all beta-lactam antibiotics except aztreonam. Aztreonam resistance in 45.7% of MBL producers could be attributed to the presence of additional resistance mechanisms.

5. CONCLUSION

Our study recorded the occurrence of MBL producers in clinical isolates of *P. aeruginosa* at 27.6% (35/127) in our setting. Azreonam was the most potent drug among MBL-producers in our study. The high prevalence of MBL-producing *P. aeruginosa* in the Enugu metropolitan is of concern because it raises the prospect of reaching a treatment impasse in the absence of newly developed therapeutic MBL inhibitors. Early identification of MBL-producing isolates is crucial for reducing infection outcomes and avoiding the spread of these strains throughout the hospital. The combined disc diffusion test (CDDT) with imipenem may be utilized as a possible screening procedure for detecting MBL production in routine microbiology laboratories where molecular methods are not possible. Continuous monitoring of MBL prevalence and the adoption of suitable antibiotic policies are critical for MBL surveillance and control in hospitals worldwide.

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